transferred to a 200 µl PCR tube. The beads were washed once with 100 µl washing buffer with LiDS and once with 50 µl washing buffer containing 100 mM Tris-HCl, pH 8.0, 0.15 mM LiCl, and 1.0 mM EDTA. (mRNA DIRECT kit.) The beads were then washed quickly with 20 µl 1x RT Buffer (25 mM Tris-HCl, pH 8.3, 37.6 mM KCl, 2.5 mM MgCl2, and 5 mM DTT) and 20 µl RT Mix containing 1X RT Buffer and 20 µM dNTP was added. The tube was heated at 65°C for 5 min. and cooled to 37°C. 1 µl MMLV reverse transcriptase (Promega) was added and the mixture was incubated at 37°C for 1 h. with occasional shaking. Next, 20 µl of water was added to the RT reaction, mixed and a 1.0 µl to 20 µl aliquot of the PCR mix containing 1x Perkin-Elmer PCR buffer, 2.0 µM dNTP, 1.0 µM T12VN, 0.2 µM arbitrary 10-mer, 1 unit AmpliTaq (Perkin-Elmer), 50 µCi α^{35}S-dATP (Amersham) was taken. PCR using temperature settings of 94°C 30", 40°C 1', 72°C 2', 40 cycles, and 72°C 10' extension was performed with the Perkin Elmer 9600 Thermal Cycler. All PCR product was run on appropriate gels for band visualization.

<u>cDNA cloning of Differential Display Bands</u>

[0164] All dried gels were marked with radioactive ink prior to film exposure for proper alignment between the X-ray film and the dried gel plate. Appropriate bands were marked by puncturing. A scalpel blade was used to score the gel around each band to be excised. The excised gel pieces were placed into a PCR tube containing 2 µl water. PCR was performed using a 50 µl PCR mix (same as for differential display with the following modifications: the primer concentration was 1 µM, and the dNTP

concentration was 200 μM; no α^{35}S-dATP is added.) The cycle settings were the same as above.

[0165] A portion of the PCR products was run on a gel to determine amount and size of PCR products; DNA that did not correspond to the size of the original differential display band was discarded. The remaining PCR fractions were purified using CHROMA SPIN-100 columns (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The purified PCR fragments were cloned into the pCR2.1 TA cloning vector (Invitrogen) according to Invitrogen cloning protocols supplied with the vector. The only variation from the standard protocol was an increase in the molar concentration of PCR product to vector (over 100-fold); multiple insertions were not found to be a problem. All ligations were performed at 16°C overnight, transformed into E. coli strain DH5α, and plated onto LB with X-gal/IPTG.

[0166] Five colonies were chosen for PCR verification; PCR products of expected size were selected. About 10 μl of the 30μl PCR reaction was simultaneously digested with *Nla* III and *Mse* I overnight at 37°C (a 5 h digestion was used as well.) cDNA clones were selected according to the colony PCR and the restriction enzyme digestion pattern.

[0167] The differential display protocol for finely staged zygotic embryos of loblolly pine as described above, has produced more than 600 differential display patterns and more than 60,000 bands. Within that set of bands, we have identified bands that increased and/or decreased during embryo development. From those bands, cDNA clones of this invention were isolated and sequenced.

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Detection of Gene Expression by Micro-Array Assay

[0168] In order to verify expression patterns of the cloned DNA in loblolly pine embryos a micro-array assay was developed. The cloned cDNAs were amplified by PCR and adjusted to equal concentrations (0.1 μg/μl). The cDNAs were then dispensed in the wells of a 384-well plate, denatured in 0.3 M NaOH at 65 °C for 30 min. and neutralized with 2 volumes of 20x SSPE mixed with 0.00125% bromophenol blue and 0.0125% xylene cyanol FF (5% gel loading dye). The denatured DNAs were then blotted on to Hybond N+ membranes (Amersham) as arrays using a VP 386 pin blotter (V&P Scientific, Inc., San Diego, CA). Each DNA was dot-blotted four times as a quartet on the membrane. An example of quartet spotting is seen in Figure 7. Each dot is about 1.2 mm in diameter and contains about 3 ng of DNA. DNA was then cross-linked to the membrane at 120,000 mJ/cm2 in a CL-1000 UV-linker. (Strategene, Inc., Upland, CA.) The dot image of each membrane was scanned, numbered and saved in computer for later use in data digitizing.

[0169] The cDNA array membranes were pre-hybridized in hybridization buffer (0.5 M Na-phosphate, pH 7.2, 5% SDS, and 10 mM EDTA) at 65°C for 30' in a hybridization oven (Model 400, Robbins Scientific, Sunnyvale, CA) and then hybridized under the same conditions with total cDNA probes made from mRNA. The membranes were washed twice at room temperature in 2x SSPE and 0.1% SDS, twice in 0.5x SSPE and 0.1% SDS, and twice in 0.1x hybridization buffer. Each wash was roughly 20 min. Each membrane was then exposed to Kodak Biomax MR films.

[0170] The total cDNA probes referred to above were made by initially creating the first strand cDNA. This was accomplished by mixing loblolly pine embryos (0.05-0.1